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Dear Tracy,

I delayed answering your letter for a few days to give myself some time to think it over since certain features of it puzzled me greatly. Let me first state that I am indeed sorry if my letter caused you any concern. I did not intend that it should. I was merely seeking information. I perhaps wrongly drew the conclusion from your Scientific American article that Lindegrenian independent experiments had been performed on the melibiose problem and was naturally anxious to find out about them. I was apparently in error. However, I was not alone in reading this implication into your statement. Several people who had not as yet taken much stock in the Lindegren statement have already communicated with me on the basis of your remark and suggested rather gleefully that this settled the issue. It may well do so in the minds of many who might have still had some doubts, but I fear that I shall have to retain some reservations. This latest development is quite typical, it seems to me, of the whole affair.

You raised certain questions in your letter and I shall try to answer them as straightforwardly and honestly as I can. If, in doing so, I appear overly harsh, please forgive me. Put it down to the intensity of my efforts to get at the truth as I see it, with perhaps some attendant sacrifice of the frills and niceties of polite intercourse. I am sure, Tracy, you would have it no other way. In the process, I shall probably raise questions of my own to which perhaps you can help provide answers. The only solutions that I have been able to entertain as reasonable have only social implications and have little to do with the scientific merits of the matter and hence I am reluctant to accept them.

You start your letter by the quotation from L. and L., C. S. H. Symposium, Vol. 11, as many other people have in recent reviews. The quotation is certainly correct although I should venture one modification, namely, instead of reading "with the help of", it should be "under the observation of" since Michael had nothing physically to do with the experiment. By their very nature he couldn't have, involving as they do crosses and microdissections. Michael's estimate of these experiments

can be objectively gauged by the fact that he was unwilling to have them published with his name attached. But actually this is beside the issue. What has surprised me is the fact that for over a year and a half now, the actual data of those experiments has been available in printed form and not one person to my knowledge, who has announced his unqualified faith in these experiments has seen fit to examine the actual results upon which his conclusions (or should I say faith) are based. I refer to the data published in Lindegren's book, *The Yeast Cell*. Now I know, Tracy, that it probably is asking a lot of any person to demand that he go through this mess in order to get at the facts of the situation. Nevertheless, it would seem to me that the usual rules of scholarly care and competence demand consideration of any existent material pertinent to arriving at an adequate estimate of the question being discussed. Particularly is this true when the material in question is the only data available and it refers to an issue as important as the continual reference to the melibiose experiment would appear to make it.

I must confess that I find it a bit surprising that people who would not believe Lindegren if he told them the time of day nevertheless accept without qualifications a remark of his on an important issue, unsupported by any experimental evidence.

In any case, let us take a look at the actual data which does exist on the question. The melibiose experiment was certainly simple in its conception. Its execution on the other hand is fraught with technical pitfalls. Some of these I did not foresee or realize at the time. In estimating the comparability or even relevance of a repetition there are many things we would like to know. One of these is the nature of the strains employed. Labels or names can be switched even in the best run laboratories dealing with thousands of strains and hence cannot be accepted as identification without question. One might hope to gain some information on the question by a careful comparison of the reported behaviour of a given (i.e., given labeled) strain under standard conditions. Even a casual reading of the Lindegren book arouses one's suspicion as to their capacity to keep their strains straight or maintain them in a condition in which they reproduce the results of a particular experiment from one period to another. There are many cases which can be cited but let me mention for an example one which is directly pertinent to the melibiose problem, since it was the first Doudoroff-observed experiment. I quote from page 10 of chapter 26. "When he (this is Mike Doudoroff) arrived, we were analyzing the matings of certain strains. Since all these haplophases had arisen from the rather regular pedigrees shown in Table 26-3 we predicted to him that only regular segregations would occur because we had finally "cleaned up" the cytoplasm. We were thoroughly surprised to find the results shown in Table 26-5." Apparently strains have a habit of suddenly changing properties in the Lindegren lab from one week to the next. Now I know

Now let us turn to the actual melibiose experiment itself which appears on page 19 of Chapter 26. It is these experiments of course which form the basis of the doubts raised by Lindegren and reechoed by others. I have discussed these very experiments in a footnote to a forthcoming paper in the Proceedings of the National Academy of Science. I am sending you a copy of this paper, but I should like to note a few things about it here. The cross which was originally employed in the melibiose experiment was one which was characterized by the fact that it invariably gave regular segregations of 2+:2- in all tetrads which were completely analyzed. I should further like to point out that this regularity did not stem merely from the "melibiose" paper itself. This cross was chosen precisely because it had exhibited complete regularity in the course of our previous investigation, published in P.N.A.S., Vol. 30, page 346. This was the paper in which we first proposed a multiple gene possibility for the inheritance of a melibiase system.

The stock used in the "repetition" does bear the number L5C, which, except for a k, does not differ from that used in the two papers to which I refer. But look at the unexpectedly different behavior under normal conditions of crossing and segregation! This cross, instead of giving the usual 2+:2- segregations which it always did in the past, yielded only one normal 2+:2- out of nine tetrads analyzed. Five of the asci yielded a 1+:3- ratio and two asci did not possess the positive allele in any of the four spores of the hybrid! The Lindegrens have by now apparently become inured to such "surprises" since no comments are made about this result. I should like to note something which you can easily verify by looking at the pedigrees we published on pages 349 and 350 of Vol. 30 of P.N.A.S. In none of our examination of the melibiose character had we ever encountered this situation; excess positives, yes, as has also Winge; but excess negatives, never. In fact, as far as I know, this type of complete "depletion" mutation only occurs in Lindegren's laboratory. Under the circumstances, with these data available, I do not think it unreasonable to entertain the supposition that the strains used by Lindegren in the "repetition" is not the one derived from the primary analysis of the melibiose pedigree and which was used in the subsequent investigation of substrate effects on segregation ratios. Or do you think me unduly harsh in the criteria I have set up for comparability? In addition, I hardly think this is the type of cross to use in an experiment designed to examine the effect of substrate on "Mendelian" segregation ratios. It is certainly stacking the cards just a wee bit if the strain can't even keep its genes straight!

We have not yet exhausted the remarkable features of this so-called experiment. If now we look at the "experimental" cross in which melibiose was maintained throughout the cycle, we find the amazing fact that an entirely different pair of spores is employed. Instead of L5C one finds L5B and instead of 20, 25. And as a matter of fact, 20 is not even a sister spore of 25 which was used in the control cross in the absence of melibiose. This is a rather queer way to try a repeat experiment of the effect of substrate on the transmission of enzymatic

capacity through meiosis. At the very least it raises serious doubts whether the experimental is comparable to the "control" cross.

Then, if we go still further and examine the results of the cross, which was done in the presence of substrate, we find amazingly enough that out of four complete tetrads analyzed, all four gave 2+:2- instead of the one out of nine obtained in the "control" cross. If therefore we do accept the experimental cross as comparable to the control, we must entertain the possibility that the presence of melibiose has had an effect on the segregation of positives since the probability that this would be due to chance would be somewhere in the neighborhood of 1 in 10^4 .

Actually of course, I don't place any faith whatsoever in these experiments in view of the way they were carried out. I do not see how they can be seriously entertained as constituting data with which the earlier results can be compared. I can make no comment on the experiments with substrates other than melibiose described in the same section as I have had no experience with them. So much for the famous repetition.

At the end of your first paragraph you point out that you "need not cite for you Winge's papers which certainly cannot be ignored in this connection, even if you criticize them." At this point I must confess you have lost me. In the first part of the same paragraph you accept Lindegren's conclusion that the melibiose experiment does not exist in fact. Then you immediately cite a paper which suggests that the facts are valid and indeed offers another explanation for them. I don't quite see how one can propose an alternative explanation and at the same time deny the validity of the facts which are presumably being interpreted. I probably am missing something here. Other people have used this combination on me. I still don't get it.

Again, I may be wrong, but in view of my analysis of the Lindegren "repetition" and Winge's findings it appears to me highly probable that we did discover in 1945 that prior contact with substrate influences the observed segregation of phenotype and that this fact was confirmed by Winge and Roberts in 1948. Certainly Winge is of this opinion as he explicitly and in no uncertain terms states on three separate occasions in the Winge-Roberts papers; once on page 310 in the fourth paragraph and again on the same page in the last paragraph and finally once more in the fifth paragraph on page 313.

After spending two years investigating his phenomenon I am almost certain he is correct and that we were dealing in the melibiose experiment with the same phenomenon. My correspondence with Winge describing our results during the course of the experiments confirms me in this opinion. I might add that Winge is beginning to wonder about cytoplasmic factors in connection with enzymatic adaptation.

In conceiving the melibiose experiments, I did not foresee the possibility of such a phenomenon as long-term adaptation and the experiments were not designed to take care of this possibility despite the fact that we held the tubes for a long period of time. From my experience in the past few years with this phenomenon, this is not the method to employ for detecting its existence.

Winge and Roberts made an extremely significant contribution but not, in my opinion, for reasons they and many others suppose. In any case as you undoubtedly have clearly seen the appearance of the Winge-Roberts paper raised serious issues with the conclusions drawn from the melibiose experiments and indeed raises doubts whether an experiment along these lines can be designed to test for the existence of gene-initiated but independently self-duplicating entities. Their results however suggest how this may be done.

In any case, I decided to devote my major efforts to an analysis of their phenomenon for I felt until the underlying basis of this was really clearly understood there was little hope of constructing definitive experiments. It was not an easy investigation and it took us almost two years of preliminary work to obtain the conditions which permitted an adequate analysis of the obvious possibilities. This time was not completely wasted for in the process we acquired the experimental "feel" for the material and phenomenon which is indispensable in biological work. It has put us in the position of being able to ask and answer questions about gene action and enzyme formation which we either did not think of or could not hope to solve prior to this work.

I have detailed these efforts a bit because in part they answer a question raised in the third paragraph of your letter on why I allowed four years to elapse without saying anything about the melibiose experiment.

In the first place I was busy doing the only thing I thought was pertinent to the issue, namely gathering some relevant facts. Perhaps I was naive about this but I felt that expressing my opinion about the adequacy of the Lindegren repetition would be rather pointless, despite the fact that I had good reasons to be suspicious. Neither I nor anybody else was in a position to estimate the validity of Lindegren's remarks of 1946 until the data upon which they were based became available and this took three years. In the meantime Winge's paper appeared in 1948 which certainly should have raised some doubts as to whether Lindegren knew what he was talking about. In my mind this last question was resoundingly settled by the appearance of his book. They prove conclusively that they haven't the foggiest notion of what an experiment is and are completely incompetent to carry one through without the closest sort of supervision.

As you well know, Tracy, I was hardly in a position in 1947 and 1948 to accede to the demand of "my public" to set up a micro-dissection lab in order to repeat the melibiose experiment. Even under the ideal conditions which exist here it has taken me a year to get such a lab built and equipped. It will take another half-year for us to become sufficiently proficient in the routine techniques to begin to put out adequate data.

And now I should like to consider the questions you posed in your postscript.

- a) Why didn't I write you when the Heredity article appeared.
- b) Why didn't I write you after seeing your course notes, which by the way are tremendous. I was delighted many times with the penetrating nature of your analysis of various problems including I might add the yeast work.
- c) Why didn't I talk to you at Columbus?

With regard to (a), please understand if the "and others" had not appeared in the Scientific American article I would not have written you about it. This term with its implications did not appear in the Heredity article. Hence in the latter you were expressing an opinion with no added information. I do not feel that I can profitably question yours or anybody else's opinion about an opinion. I must confess I was surprised that in the Heredity article you did not consider the data existent in Lindegren's book. But what with time lag between writing and publishing I conceived it possible that it was not yet available to you at the time of writing.

With regard to (b); this I find the most surprising of all. Except for details I am in complete agreement with your discussion of the data you consider on yeast in your notes. I don't have the notes available now since I returned them some time ago, but as I recall, you very correctly pointed out that in view of Winge's results and interpretations it was difficult to understand why Lindegren could not repeat the melibiose experiment. You raised questions of whether the experiments had been properly performed, whether they had been adapted sufficiently. Between the time of writing these notes and your review articles you have apparently satisfied yourself that these reservations, and the apparent contradictions between Winge and Roberts and Lindegren no longer existed or were not crucial to the discussion.

With regard to (c), rightly or wrongly I got the rather strong impression from our brief contacts at Columbus that you were preoccupied with many things at Columbus. I felt it highly unlikely that either one of us could have profited from a discussion in the atmosphere that prevailed there. The few frequently interrupted exchanges we did have did not encourage me to entertain any other view. It was for that reason that I suggested that I pay you a visit in which we could go over our researches in a more relaxed and rational environment.

Well, I must bring this letter to a close. Before I do so there are several remarks I would like to make. It may indeed be probable that in the 78 or so papers that I have published mistakes have been made. I do not fear the committing of some in the future. I have never considered that the proper functions of any member of society, and least of all a scientist, should be the jealous guarding of his reputation to the end that when he dies it may be said of him "he never had to retract either a theory or a fact." This kind of epitaph can be written for a multitude made mute by virtue of lack of ability or otherwise, but can apply to relatively few who, by their creative efforts, have fruitfully enlarged our knowledge and understanding of nature. If I make an error I will admit it. However, I will not let fear of committing one paralyze me into inactivity. But I will be damned if I will seek salvation from the Gods of genetics (or any others) by confessing sins they presume I have committed.

This has been a painful letter to write and took more time and effort than even its inordinate length would indicate. It has taken me over ground I have traversed many times fruitlessly in the past four years. I am always led back to the rather bitter conclusion that it would have been better for the field had I never conceived and performed an experiment I had looked upon with some pride. It has engendered only irrationality and confusion and all for heartbreakingly irrelevant reasons of incompetence.

I hope my efforts will be of some help to you in arriving at an understanding of my views. If not, they will have been doubly in vain. I do not expect to persuade you to my viewpoint by this. Issues such as these are not solved by such letters or semi-popular articles or "corrective" letters to the editors. Those aspects worthy of our attention will be resolved only by the accumulation of more information and the design of the proper experiments. This I am trying to do to the best of my ability as I know you are. Our job is not made easier by the background noise.

Let me end by saying this, Tracy, you are one of the very few men in American Biology of whom I think highly enough to write all I have included in this letter.

Depressedly yours,

S. Spiegelman

SS:mb